(12)

DNA-TR-86-155

THE PROLIFERATIVE CHARACTERISTICS OF INTESTINAL STEM CELLS

Response and Protection to High Energy or Fission Spectrum Neutrons or Photons

Wayne R. Hanson
Rush-Presbyterian-St. Luke's Medical Center
1753 West Congress Parkway
Chicago, IL 60612

30 April 1986

Technical Report

CONTRACT No. DNA 001-84-C-0061

THIS WORK WAS SPONSORED BY THE DEFENSE NUCLEAR AGENCY
UNDER RDT&E RMSS CODE B344082466 Y99QAXSG00048 H2590D.

Prepared for
Director
DEFENSE NUCLEAR AGENCY
Washington, DC 20305-1000

DISTRIBUTION LIST UPDATE

This mailer is provided to enable DNA to maintain current distribution lists for reports. We would appreciate your providing the requested information.

☐ Add the individual listed to your distribution list.

☐ Delete the cited organization/individual.

☐ Change of address.

NAME: _____

ORGANIZATION: _____

OLD ADDRESS	CURRENT ADDRESS
_____	_____
_____	_____
_____	_____

TELEPHONE NUMBER: ( ) _____

SUBJECT AREA(s) OF INTEREST:

_____	_____
_____	_____
_____	_____

DNA OR OTHER GOVERNMENT CONTRACT NUMBER: _____

CERTIFICATION OF NEED-TO-KNOW BY GOVERNMENT SPONSOR (if other than DNA):

SPONSORING ORGANIZATION: _____

CONTRACTING OFFICER OR REPRESENTATIVE: _____

SIGNATURE: _____

Director
Defense Nuclear Agency
ATTN: STTI
Washington, DC 20305-1000

Director
Defense Nuclear Agency
ATTN: STTI
Washington, DC 20305-1000

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-A176675

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY N/A since Unclassified	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE N/A since Unclassified	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) DNA-TR-86-155
6a. NAME OF PERFORMING ORGANIZATION Rush-Presbyterian-St. Luke's Medical Center 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION Director Defense Nuclear Agency
6c. ADDRESS (City, State, and ZIP Code) 1753 West Congress Parkway Chicago, IL 60612	7b. ADDRESS (City, State, and ZIP Code) Washington, DC 20305-1000
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DNA 001-84-C-0061
8c. ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62715H; PROJECT NO. Y99QAXS; TASK NO. G; WORK UNIT ACCESSION NO. DH008095

11. TITLE (Include Security Classification)
THE PROLIFERATIVE CHARACTERISTICS OF INTESTINAL STEM CELLS
Response and Protection to High Energy or Fission Spectrum Neutrons or Photons

12. PERSONAL AUTHOR(S)
HANSON, Wayne R.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical	FROM 831201 TO 851201	860430	32

16. SUPPLEMENTARY NOTATION
This work was sponsored by the Defense Nuclear Agency under RDT&E RMSS Code B344082466 Y99QAXSG00048 H2590D.

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
6	18	
20	8	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Radiation Protection; Proliferative Response; Stem Cells; Gastrointestinal System; Cytosine Arabinoside; Ara/c; WR-2721

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Cytosine Arabinoside (Ara/c) is an S phase cytotoxic agent. Since nearly half the proliferating cells in the murine crypt are in the S phase, Ara/c kills 50% of this cell population. However, 12 hours after Ara/c treatment, the clonogenic cells (the cells responsible for tissue regeneration) in the crypt are considerably less sensitive to photon radiation than clonogenic cells of control animals. Evidence suggests that the reason for this radioprotection by a toxic agent is the Ara/c-induced alteration in the cell age distribution of the clonogenic cells. Normally, the clonogenic cells are in a[illegible] or G2 stage of the cell cycle and are unaffected directly by Ara/c; however, following Ara/c treatment of an animal, the clonogenic cells enter the cell cycle. By 12 hours, the clonogenic cells proceed in a partially synchronized fashion to a mid to late S phase of the cell cycle where they are less sensitive. WR-2721 appears to protect cells from radiation throughout the cell cycle and most likely acts through a mechanism different

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a NAME OF RESPONSIBLE INDIVIDUAL Betty L. Fox	22b TELEPHONE (Include Area Code) (202) 325-7042 22c OFFICE SYMBOL DNA/STTI

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

18. SUBJECT TERMS (Continued)

Fermilab High Energy Neutrons
JANUS Fission Spectrum Neutrons

19. ABSTRACT (Continued)

than Ara/c. Results of this contrast showed that the combination of Ara/c and WR-2721 protected the gut from photon injury to a greater extent than each agent alone. The protection from Fermilab neutrons by the combination was slightly better than each agent and there was no additional protection of Ara/c combined with WR-2721 for injury by JANUS fission spectrum neutrons. These treatments did not alter the animal response at doses in the bone marrow lethal range. These data are consistent with findings that the intestinal clonogenic cell age distribution has less effect on high LET radiosensitivity. The results of experiments funded by this contract show that the intestinal stem cells are normally in a G_0 stage of the cell cycle and respond to Ara/c by entering the cell cycle at the S phase in which they show a pronounced change in radiosensitivity to photon injury. These studies are part of the overall goal to research mechanisms of intestinal protection from photon and neutron injury.

CONVERSION TABLE

Conversion factors for U.S. Customary to metric (SI) units of measurement.

MULTIPLY ⟶ BY ⟶ TO GET
TO GET ⟵ BY ⟵ DIVIDE

angstrom	1.000 000 X E -10	meters (m)
atmosphere (normal)	1.013 25 X E +2	kilo pascal (kPa)
bar	1.000 000 X E +2	kilo pascal (kPa)
barn	1.000 000 X E -28	meter^2 (m^2)
British thermal unit (thermochemical)	1.054 350 X E +3	joule (J)
calorie (thermochemical)	4.184 000	joule (J)
cal (thermochemical)/cm^2	4.184 000 X E -2	mega joule/m^2 (MJ/m^2)
curie	3.700 000 X E +1	giga becquerel (GBq)*
degree (angle)	1.745 329 X E -2	radian (rad)
degree Fahrenheit	$t_K=(t°f+459.67)/1.8$	degree kelvin (K)
electron volt	1.602 19 X E -19	joule (J)
erg	1.000 000 X E -7	joule (J)
erg/second	1.000 000 X E -7	watt (W)
foot	3.048 000 X E -1	meter (m)
foot-pound-force	1.355 818	joule (J)
gallon (U.S. liquid)	3.785 412 X E -3	meter^3 (m^3)
inch	2.540 000 X E -2	meter (m)
jerk	1.000 000 X E +9	joule (J)
joule/kilogram (J/kg) (radiation dose absorbed)	1.000 000	Gray (Gy)**
kilotons	4.183	terajoules
kip (1000 lbf)	4.448 222 X E +3	newton (N)
kip/inch^2 (ksi)	6.894 757 X E +3	kilo pascal (kPa)
ktap	1.000 000 X E +2	newton-second/m^2 ($N-s/m^2$)
micron	1.000 000 X E -6	meter (m)
mil	2.540 000 X E -5	meter (m)
mile (international)	1.609 344 X E +3	meter (m)
ounce	2.834 952 X E -2	kilogram (kg)
pound-force (lbf avoirdupois)	4.448 222	newton (N)
pound-force inch	1.129 848 X E -1	newton-meter (N·m)
pound-force/inch	1.751 268 X E +2	newton/meter (N/m)
pound-force/foot^2	4.788 026 X E -2	kilo pascal (kPa)
pound-force/inch^2 (psi)	6.894 757	kilo pascal (kPa)
pound-mass (lbm avoirdupois)	4.535 924 X E -1	kilogram (kg)
pound-mass-foot^2 (moment of inertia)	4.214 011 X E -2	kilogram-meter^2 ($kg·m^2$)
pound-mass/foot^3	1.601 846 X E +1	kilogram/meter^3 (kg/m^3)
rad (radiation dose absorbed)	1.000 000 X E -2	Gray (Gy)**
roentgen	2.579 760 X E -4	coulomb/kilogram (C/kg)
shake	1.000 000 X E -8	second (s)
slug	1.459 390 X E +1	kilogram (kg)
torr (mm Hg, 0°C)	1.333 22 X E -1	kilo pascal (kPa)

* The becquerel (Bq) is the SI unit of radioactivity; 1 Bq = 1 event/s.
**The Gray (Gy) is the SI unit of absorbed radiation.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

SECTION 1

INTRODUCTION

The intestinal cell renewal system in mammals is one of the most radiosensitive systems in the body and constitutes a dose-limiting organ. Animals and man exposed to whole body radiation doses above 15 Gy photons or about 5 Gy fission spectrum neutrons die from a complicated gastrointestinal syndrome associated with diarrhea, malabsorption, bacterial sepsis and the loss of intestinal stem cell reproductive integrity which leads to mucosal collapse (1). The actual cause of death of the organism has not been associated with any one specific effect and is most likely a response to the perturbation of tissue function associated with the collapse of structural integrity. In turn, the structural integrity of the tissue after radiation is dependent, to a large degree, on the survival and proliferation of intestinal stem cells; those cells which reproduce their own population and give rise eventually to the differentiated absorptive villus columnar epithelium. The survival of intestinal stem cells from radiation and the subsequent proliferation of these cells is the basis of the microcolony assay (2). The basic assumptions attendant to this assay are; 1) a single surviving cell gives rise to a single microcolony, 2) clonogenic (stem) cell survival is independent, that is, the survival of one cell will not enhance or decrease the ability of another cell to survive, (this is an assumption associated with clonogenic assays in general and, at this point, there is no evidence to the contrary), 3) the rapidly cycling cells within the crypt are the stem cells (3-7). Presumptive evidence for the third and perhaps most far-reaching assumption comes from the argument in the original paper by Withers and Elkind (3) describing the assay. The authors suggested that in order for a multicellular epithelial foci to form by 4 days, the surviving cells must proliferate rapidly and therefore, must come from cells having a cell cycle time of about 11-12 hours. This is the cell cycle time of the rapidly cycling crypt cell population (8). A more sophisticated argument that intestinal stem cells are rapidly cycling in the normal animal comes from the split dose experiments of Masuda et al. (6) who showed that the extrapolation numbers between a single dose survival curve and the split dose curve suggested that there were about 140 stem cells per crypt; a value consistent with the rapidly cycling cell population within each crypt.

The interpretation of results gathered up to 1975 suggested that the intestinal cell renewal system was fundamentally different than other cell systems such as the hematopoietic or epidermal steady state systems which have a slowly cycling small stem cell compartment which feeds into a rapidly cycling amplification compartment. In contrast, Potten and Hendry (9) carefully analyzed the kinetics of regeneration of intestinal mucosa after irradiation and argued that mucosal regeneration appeared to come from a small population of stem cells, probably in the base of the crypts, which have the subcellular organization associated with a more primitive undifferentiated cell (10,

11). These results were the impetus for experiments devised in our laboratory to test the hypothesis that the intestine has a small, slowly cycling stem cell population and that this population is responsible for the regeneration of the mucosa following irradiation. In the initial studies, high specific activity tritiated thymidine (HSA ^{3}HTdR) was given either at 1 hour or at both 1 and 17 hours before irradiation with graded doses of ^{60}Co for the microcolony assay (12). Crypt damage from the HSA 3-HTdR was measured be dissecting, squashing, and counting the total number of cells per crypt using the method of Wimber et al. (13). Crypt damage was assayed in some animals at the same time other similarly treated animals were given a microcolony assay dose of ^{60}Co. The HSA ^{3}HTdR reduced crypt cellularity from 250 cells to 140 cells within about 6 hours, however, the cell survival curves were identical. These results suggested that there were few intestinal clonogenic stem cells in the S- phase of the cell cycle and supported the results of Potten et al. (9) showing a separate stem cell population. Further supportive evidence came from experiments using prolonged colcemid treatment to selectively kill rapidly cycling cells in the crypt (12). A summary of the experimental design is shown in Figure 1.

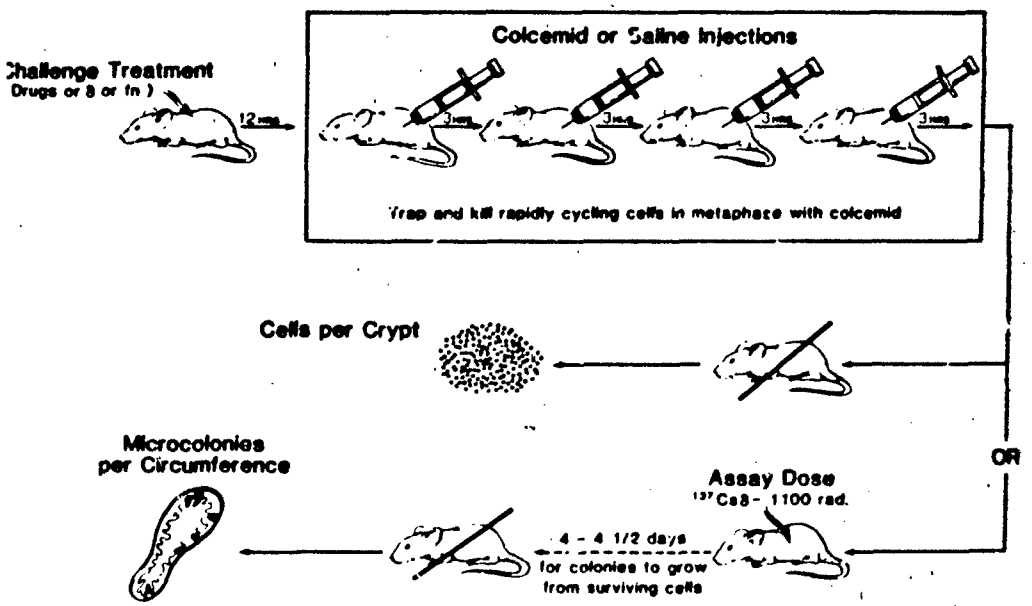

Figure 1. Experimental design of studies using long term colcemid block to selectively kill rapidly cycling cells.

Colcemid, a staphmokinetic agent, prevents the formation of a spindle apparatus and thus prevents anaphase development. Rapidly cycling crypt cells blocked in metaphase began to lyse

after 3 hours. By giving colcemid (150 ug/mouse) every 3 hours for 12 hours (4 IP injections), the crypt cell number was reduced from 250 to about 130. Animals treated with 12 hours of colcemid, however, have clonogenic survival curves identical with control animals having 250 cells per crypt. Prolonged colcemid treatment had no apparent effect on the clonogenic population.

In view of our results which have recently been confirmed (14), the proliferative characteristics of the intestinal stem cells that survive radiation is important to investigate. If the stem cells are normally few in number and not in rapid cycle before irradiation; then, the stem cells must be recruited into rapid cycle to produce a large foci of epithelium 4 days later. To determine if radiation could recruit the stem cells into rapid cycle, doses of 0.5 to 2.5 Gy ^{137}Cs were given to mice, whole body, and at 6 hour intervals after this challenge dose of radiation, a 12 hour treatment of colcemid was given. At the end of the colcemid treatment, a single large assay dose of ^{137}Cs was given for the microcolony assay. The experimental design outlined in Figure 1 was used. The results shown in Figure 2 shows that recruitment occurred and the the greatest number of stem cells were killed when colcemid was given beginning 12 hours after the challenge dose of radiation. These results are consistent with the hypothesis that the stem cells are normally few in number and slowly proliferating until recruited into cycle.

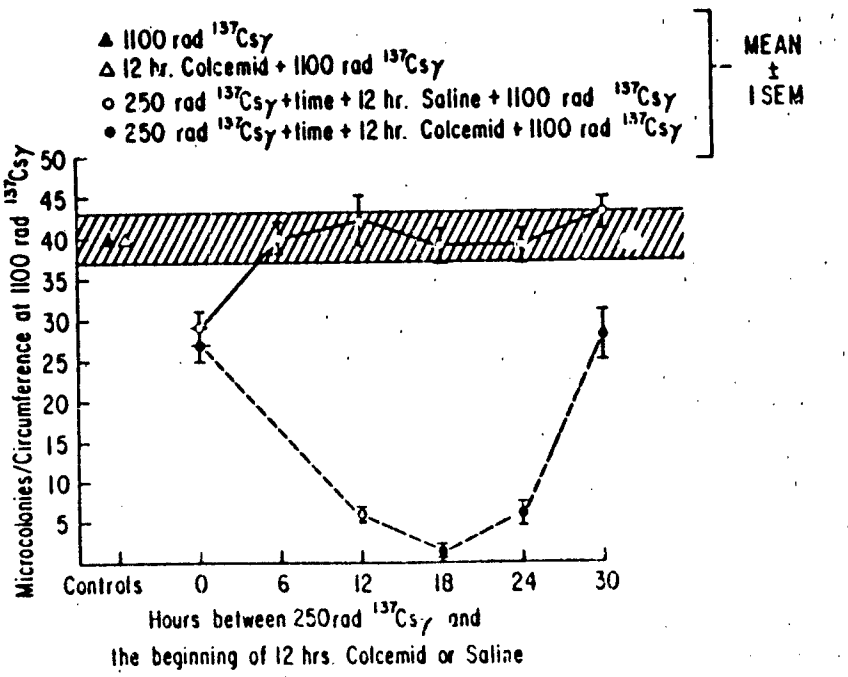

Figure 2. Jejunal microcolonies at a single dose of 11.0 Gy ^{137}Cs in control animals, in animals given 12 hrs colcemid, or in animals given a challenge dose of 2.50 Gy ^{137}Cs before 12 hrs of saline or 12 hrs of colcemid begun at increasing times after the challenge.

Radiation is only one of several cytotoxic agents that appears to recruit stem cells from a very slow cycle into a rapid cycle. Our experience has shown that cytotoxic drugs such as Hydroxyurea (HU) (15) and Cytosine Arabinocide (Ara/c) (16) also exert a potent stimulus for intestinal stem cells to enter the cell cycle. Phelps (17) showed that the post Ara/c radiosensitivity of the stem cells was considerably less at 12 hours after Ara/c administration. These results have been confirmed and extended in our laboratory to show that the likely mechanism for the reduced radiosensitivity is an altered proliferative state of the intestinal stem cells. At 12 hours after Ara/c, a sizable portion of stem cells appear to be held up in the less radiosensitive mid to late S portion of the cell cycle. Ara/c is a potent S-phase cytotoxic agent and kills about 90 crypt cells. When Ara/c was followed be 12 hours of colcemid, the total crypt population was reduced to about 80 cells, however, the intestinal stem cells were still radioresistant compared to controls. Therefore, the radioprotection afforded to the intestinal stem cells by Ara/c appears to be through the recruitment of these cells into the more radioresistant mid to late S phase of the cell cycle.

Radioprotection by the thiol compounds are by a different mechanism. The most widely studied and effective IN VIVO radioprotectant among the thiol agents is S-2-'3-aminopropylamino) ethophosphorothioxic acid (WR-2721). The mechanisms of radioprotection of thiol compounds are most likely by hydrogen atom donation, competition of sulfhydryl groups with free radicals (free radical scavenging), or by the induction of hypoxia.

The mechanisms of radioprotection by the two types of agents (recruiting agents and thiol compounds) appeared to be different, therefore studies were undertaken to determine if the combination of these types of agents were additive in their radioprotective effect when given before photons and two types of neutron radiation.

SECTION 2

MATERIALS AND METHODS

Specific Aim 1: To measure clonogenic stem cell survival in control animals and in animals treated with Ara/c, WR-2721, and the combination of both, given before ^{137}Cs gamma-rays, Fermilab neutrons or JANUS neutrons.

Aim 1a: Radioprotection of intestinal clonogenic stem cells from ^{137}Cs gamma-rays.

Male C3H/HeJ mice (Jackson Laboratories, Bar Harbor, Maine) were housed in the animal quarters at Rush University until they were 100-130 days old. Mice were divided into four main groups; controls (given saline), Ara/c treated (250 mg/Kg), WR-2721 (12 mg/30 g mouse) or the combination of the two agents. The Ara/c treated animals were given graded doses of ^{137}Cs irradiation 12 hours after IP administration of Ara/c, the time which has been shown to yield the greatest radioprotection. The WR-2721 treated animals were similarly irradiated 30 min after IP administration which has been shown to be most effective. In the combination treated animals, the Ara-c was given 12 hours before and the WR-2721 was given 30 min before irradiation. Four days after irradiation, the animals were given 25 uCi tritiated thymidine ($^{3}HTdR$) IP and killed 1 hour later. The jejunum was fixed in alcohol, formalin, and acetic acid (AFA, 20:2:1), embedded in paraffin, and sectioned for histology. Autoradiographs were prepared by the dipping technique using Kodak NTB emulsion. The dipped slides containing 5 u cross-sections of jejunum were stored at 4° C for 10 days, then developed in Kodak D-19 developer before they were stained with hematoxylin. After staining, the number of microcolonies were counted per circumference and plotted versus dose of radiation for each of the treatment groups. At the time the animals were killed, a second portion of jejunum was fixed in Carnoy's fixative for the crypt squash preparations as described by Wimber et al. (13).

Aim 1b: Radioprotection of intestinal clonogenic stem cells from Fermilab neutrons.

Male C3H/HeJ mice (100-140 days old) were given Ara/c (250 mg/Kg) or saline and transported to Fermilab so that the animals could be irradiated with neutrons 12 hours later. The neutron beam at Fermilab is produced by a 66.67 MeV proton beam on a "50 MeV" beryllium target with a gold exit face (18). Doses of high energy neutrons (average energy of the fermilab beam is 25 MeV) between 6.5 and 12.0 Gy were given to groups of control mice, mice treated with Ara/c 12 hrs before irradiation, mice treated with WR-2721 one hr before irradiation , and a fourth group treated with the combination of the two agents, Ara/c 12 hrs before and WR-2721 one hr before irradiation. The high energy neutrons were delivered in the following way: unanesthetized animals were placed in perforated tubes held by elastic bands on

a tissue equivalent plastic disk of shonka A-150 material (0.854g/square cm), sufficiently thick for build-up. A second A-150 disk of similar thickness for radiation backscatter was placed such that the mice were sandwiched be the two disks. This assembly was aligned by fixed laser beams which were vertical and perpendicular to the port and 150 cm from the beryllium target. The neutron beam diameter at this distance was about 30 cm with a dose variance of $\pm$ 3 %. An EXRADIN air-filled ionization chamber, made with Shonka A-150 tissue equivalent plastic walls were used for neutron dosimetry. Neutron flux monitors immediately distal to the target were used to calibrate the beam relative to the ionization chamber measurements. The total Fermilab neutron dose were computer controlled by the flux monitor readings. The neutron doses reported include a gamma component estimated to be about 5-7%. The neutron doses were delivered 12 hrs after Ara/c administration. This time has been shown to produce the maximum protective effect (14,17). WR-2721 was dissolved in phosphate buffered ringers at a pH of 6.8 and given 1 hr before irradiation IP at a dose of 500 mg/Kg. Sigdestad et al. have shown this dose and time interval to be the most effective (19). After irradiation, the mice were taken back to the Rush-Presbyterian animal facilities and four days later, they were given an IP injection of ^{3}HTdR (1 uCi/g body weight) and killed 1 hr later. Autoradiographs of intestinal cross-sections were examined and the number of microcolonies per circumference were assayed as described and plotted versus Fermilab neutron dose.

Aim 1c. Radioprotection of intestinal clonogenic stem cells from JANUS neutrons.

Male C3H/HeJ mice were purchased from Jackson laboratories and sent directly to Argonne National Laboratory. After an appropriate quarantine time, the animals were divided into the same groups as described above: controls, Ara/c given 12 hrs before irradiation, WR-2721 given 1 hr before irradiation, and the combination given at the same times as each agent alone. Irradiations were done by placing the treated mice into plastic cups which were, in turn, placed along isodose lines within the high flux area of the JANUS research reactor (20). The dose rate was 15 cGy per min with a gamma component of about 3%. Four days following irradiation, the mice were injected with ^{3}HTdR and killed one hr later for the microcolony assay as described.

Specific Aim 2: To estimate the LD50/$_6$ and LD50/$_{30}$ in controls, Ara/c treated mice, WR-2721 treated mice. and in mice given the combination of the two agents before ^{137}Cs, Fermilab neutrons, or JANUS neutrons.

The same four treatment groups as described above (control, Ara/c 12 hrs before irradiation, WR-2721 one hr before irradiation, or Ara/c 12 hrs before and WR-2721 one hr before irradiation) were used to estimate both the LD50/$_6$ associated with intestinal death, and the LD50/$_{30}$ associated with bone marrow death in mice irradiated with ^{137}Cs, Fermilab neutrons, or JANUS neutrons.

The mice irradiated with gamma-rays at Rush were housed in the Rush animal quarters and checked twice daily [LD50/$_6$ dose range], or once daily [LD50/$_{30}$ dose range]. The data were collated on the 6th day or the 30th day.

The mice to be irradiated with Fermilab neutrons were ferried to the neutron treatment facility in a car, then returned to the Rush animal facility where they were checked at the appropriate times.

Mice irradiated with JANUS neutrons were housed at Argonne National Laboratory animal facilities where they were kindly checked for mortality by Ms Jane S. Heulsch.

SECTION 3

RESULTS

Aim 1a: Radioprotection of intestinal clonogenic stem cells from ^{137}Cs gamma-rays.

The clonogenic stem cell survival curve for the control animals was similar to that found previously (12), Figure 3. The D_o was 1.56 ± 0.13 Gy. At 12 hours following a single injection of Ara/c, the D_o was similar to the control value (1.64 ± 0.15 Gy); however, the shoulder of the survival curve was increased (figure 3). WR-2721 increased both the shoulder and the slope of the clonogenic survival curve. The D_o was 1.96 ± 0.19 Gy. The greatest radioprotection was seen when the combination of treatments was given, Figure 3.

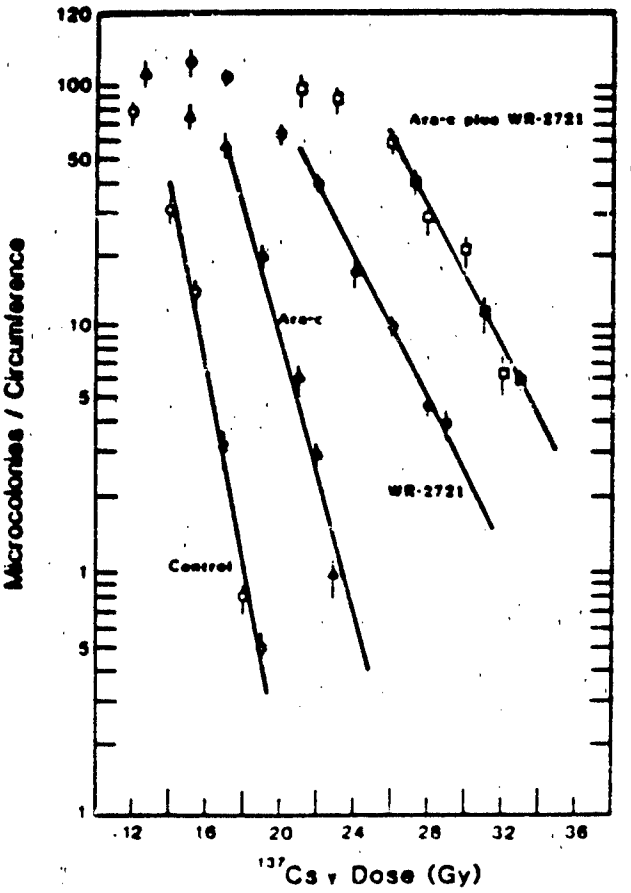

Figure 3. Jejunal microcolonies per circumference in control mice or in mice given Ara/c 12 hours before, WR-2721 1 hour before, or the combination of the two agents; Ara/c 12 hours and WR-2721 1 hour before irradiation with graded doses of ^{137}Cs.

The shoulder was greatly increased; however, the slope remained about the same as in the animals treated with WR-2721 alone. The D_o in the combination treated animals was 1.87 ± 0.14 Gy.

The radioprotection shown in the Ara/c treated animals was at a time when there were only about 170 cells remaining out of the normal 250 cells per crypt in untreated animals as shown in Figure 4. This reduction in cells in Ara/c treated animals was consistent with the S-phase cytotoxicity of the drug.

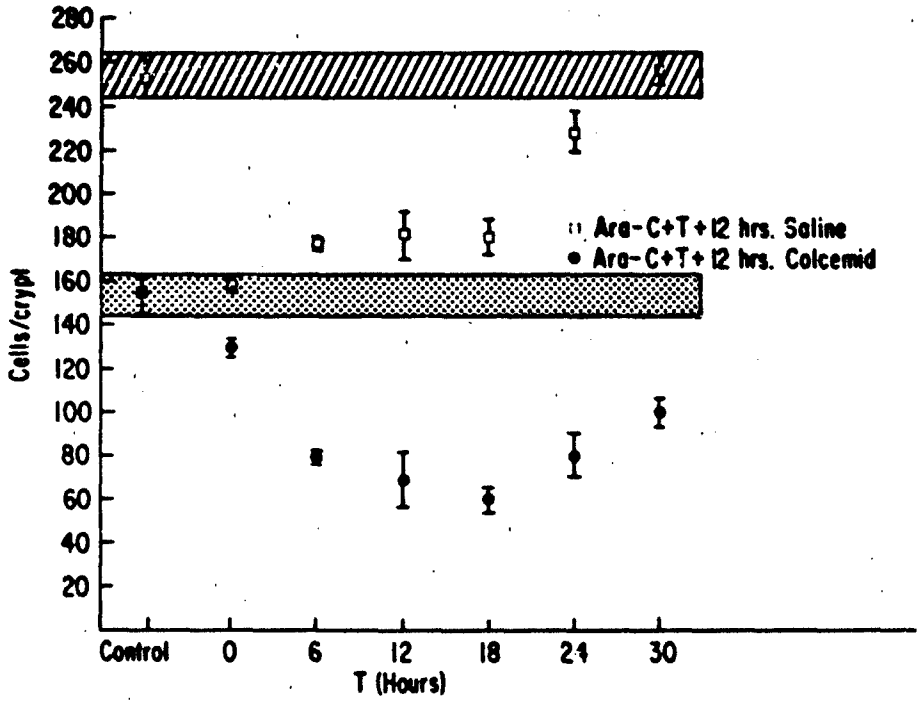

Figure 4. Number of cells per crypt in control (untreated) animals or at various times after Ara/c in animals given 12 hours saline of 12 hours colcemid block.

It is of interest to note that the animals given the colcemid had crypts consisting of only about 70 cells and yet the radiation survival curve showed the same degree of radioprotection as in the animals given Ara/c alone. These data are a strong demonstration that the rapidly cycling cells are not the clonogenic population.

Aim 1b: Radioprotection of intestinal clonogenic stem cells from Fermilab neutrons.

The clonogenic cell survival after Fermilab neutrons is shown in Figure 5.

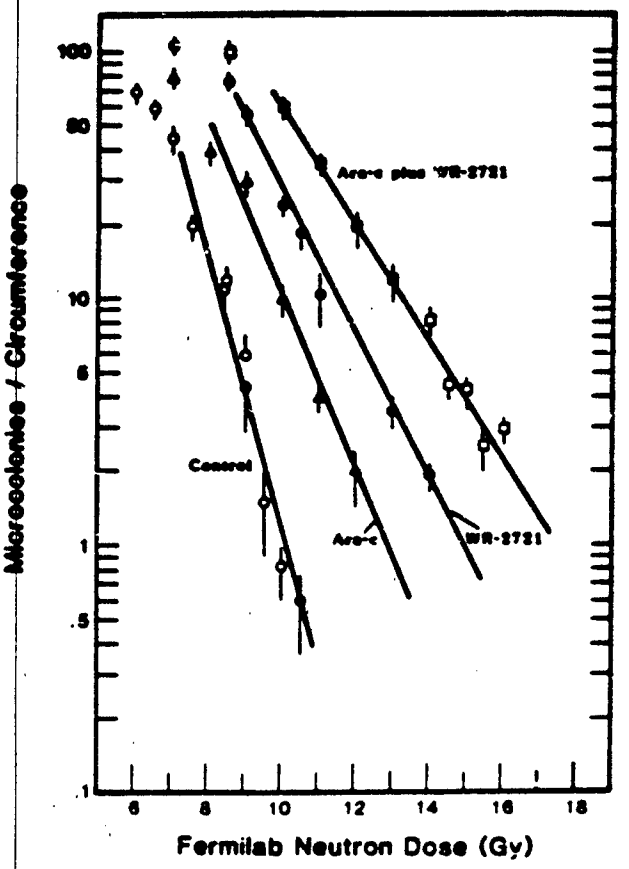

Figure 5. Jejunal microcolonies per circumference in control animals, or in animals treated with Ara/c 12 hours before, WR-2721 one hour before, or the combination; Ara/c 12 hours before and WR-2721 1 hour before graded doses of Fermilab neutrons.

The control curve had a D_o of 1.05 ± 0.13 Gy. The D_o was increased at 12 hours after Ara/c; however, the shoulder was the feature of the cell survival curve that was changed the most. The D_o was increased to 1.27 ± 0.12. WR-2721 increased the shoulder even more, and the D_o was increased to 1.35 ± 0.16 Gy. As with the drug-induced changes in the gamma survival curves, the combination of agents altered the curve the most in Fermilab irradiated animals. The shoulder of the curve was extended and the D_o was increased to 1.54 ± 0.18 Gy.

Aim 1c: Radioprotection of intestinal clonogenic stem cells from JANUS neutrons.

The effect of the high LET, low energy fission spectrum neutrons from the Argonne JANUS reactor on animals given Ara/c and/or WR-2721 in the same regimen as outlined above is shown in Figure 6.

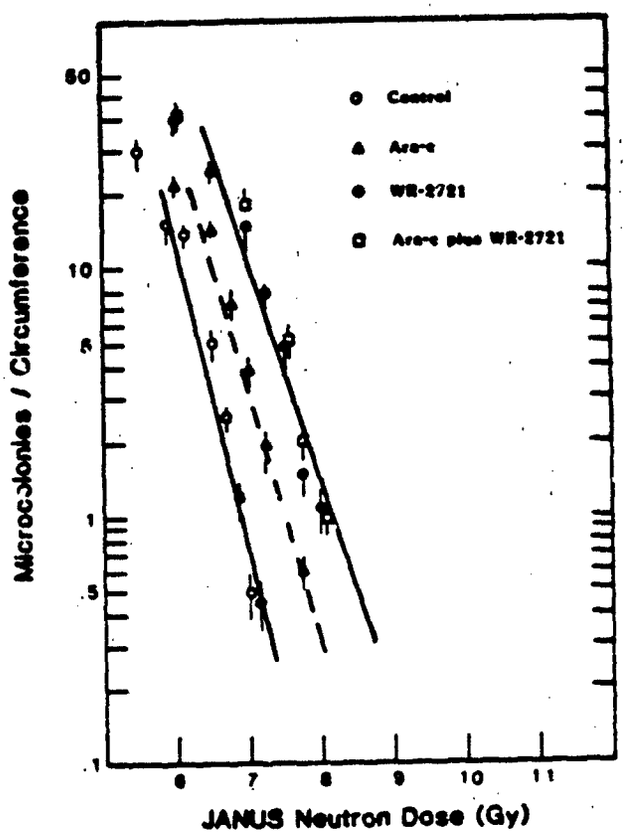

Figure 6. Jejunal microcolonies per circumference in control mice or in mice treated with Ara/c 12 hours before, WR-2721 1 hour before, or the combination of the two agents; Ara/c 12 hours and WR-2721 1 hour before irradiation with graded doses of JANUS neutrons.

The changes were less than those seen for the other types of radiation. The D_0 for the control animals was 0.53 ± 0.07. The Ara/c regimen increased the shoulder of the curve; however, the D_0 remained about the same at 0.62 ± 0.07 Gy. WR-2721 increased the shoulder but the D_0 did not change: 0.59 ± 0.09 Gy. The combination of the two agents did not alter clonogenic survival compared to cell survival after WR-2721 alone.

Aim 2: The estimate of the $LD50/_6$ and $LD50/_{30}$ in controls, Ara/c treated mice, WR-2721 treated mice, and in mice given the combination of the two agents before ^{137}Cs, Fermilab neutrons or JANUS neutrons.

The LD50/6 was used to measure mortality in this strain of mice, rather than the more conventional LD50/3 since the 6 day time period appears to better reflect death due to the gastrointestinal syndrome. The results of the Ara/c and/or WR-2721 given in the regimens outlined above are shown in Table 1.

Table 1. The mean ^{137}Cs, JANUS neutron, or Fermilab neutron dose (Gray) resulting in 6 day mortality of 50% of a population of C3H/HeJ mice in controls or in mice treated with Ara/c, WR-2721 or the combination of both.

	LD50/6		
	^{137}Cs	FERMI NEUTRONS	JANUS NEUTRONS
CONTROL	16.95 (16.61-17.28)*	8.75 (8.43-9.07)	4.0 (3.31-4.69)
Ara/c	17.6 (16.40-17.63)	9.21 (8.91-9.5)	3.9 (3.24-4.72)
WR-272	25.24 (25.13-25.34)	15.0 (13.52-16.48)	4.73 (3.40-5.07)
Ara/c plus WR-2721	25.13 (24.87-25.39)	15.0 (14.26-15.74)	4.66 (4.35-4.97)

* 95% Confidence Limits

The control LD50/6 values for the different radiations were similar to those reported previously (5,7). Ara/c had only a small effect and increased the LD50/6 value slightly. WR-2721 had a much greater protective effect, but that effect was greatest for the low LET gamma irradiation as was expected. The protective effect was the least for JANUS neutrons and was intermediate for Fermilab neutrons. The protective factors calculated from Table 1 are given in Table 2.

Table 2. The dose modification (protection) factors for LD50/6 calculated from Table 1 for mice treated with the different drug regimens.

	PROTECTION FACTORS		
	LD50/6		
	^{137}CS	FERMI NEUTRONS	JANUS NEUTRONS
CONTROL	1.0	1.0	1.0
Ara/c	1.04	1.05	1.0
WR-2721	1.5	1.7	1.2
Ara/c plus WR-2721	1.5	1.7	1.2

SECTION 4

DISCUSSION

The most effective exogenous radioprotectants from photon radiation injury in mammals are the thiol compounds developed by the Experimental Therapeutics division at Walter Reed Medical Center (21). The most widely studied and effective IN VIVO radioprotectant among these is WR-2721. The mechanisms of radioprotection of thiol compounds are most likely by hydrogen atom donation (22), competition of sulfhydryl groups with free radicals (free radical scavenging) or by the induction of hypoxia (23). These mechanisms are attributed, in general, to radioprotection by compounds containing thiols, both exogenously administered or endogenous thiol compounds such as glutathione (24,25).

The radioprotection of intestinal clonogenic cells by Ara/c has not been widely studied and the mechanism is unknown. It is particularly interesting that such a potent S-phase cytotoxic agent can protect intestinal stem cells from both photon and neutron irradiation. The most likely explanation is the recruitment of G_0 stem cells into rapid cycle (26).

It is widely accepted that the stem cells of the bone marrow and the skin are normally in a very long G_1 or in a G_0 stage of the cell cycle out of which a few cells may enter the rapidly cycling pool of cells. After dividing, one of these cells may enter the differentiating amplification compartment and the other may remain a stem cell. Until several years ago, it was believed that the intestinal cell renewal system was fundamentally different. It was thought that the rapidly cycling cells in the lower two-thirds of the crypt were equivalent to the stem cells, and that by killing these cells with radiation or with cytotoxic drugs, the number of cells which could give rise to the regenerative foci of intestinal epithelial cells to repopulate the mucosa would be recruited. Several experiments in our laboratory showed that this was not true (12,15). Reduction of the rapidly cycling cells in the crypts by about half did not reduce the number of clonogenic cells. These findings were confirmed in the studies reported here. Ara/c reduced the number of cells in the crypt and by 12 hours later, there was less than half the normal number of cells/crypt; yet at this time post-Ara/c treatment, the number of surviving clonogenic cells was greatly increased. These results strongly suggest that the rapidly cycling cells within the crypts are not the clonogenic cells. This same conclusion has now been reached by other investigators (14). This alone does not explain why Ara/c protects intestinal clonogenic cells from radiation injury. Radioprotection must come from the redistribution of the clonogenic cells within the cell cycle. Specifically, Ara/c must recruit the stem cells into rapid cycle where they are less radiosensitive. At 12 hours after Ara/c, the stem cells must be in mid to late S-phase. The effect of this redistribution on the clonogenic cell survival curve was an increase in the shoulder of the survival curve, but little effect on the slope of the curve. The shoulder of the intestinal survival curve is attributed to repair of sublethal damage (SLD)

and to multiplicity (the number of stem cells per crypt). It has been shown that the multiplicity does not change during the 12 hours after Ara/c (16). These results suggest that increased repair capability may exist in these mid to late S-phase cells. Qualitatively similar results were found in Ara/c treated animals irradiated with the two neutron sources. The least effect of Ara/c was found in animals irradiated with JANUS neutrons and the results from Fermi irradiated animals were intermediate. The reduced cell age effect of neutron irradiation has been documented previously (27).

In contrast to Ara/c, WR-2721 increased both the shoulder and the slope of the intestinal clonogenic cell survival curves. The shoulder was increased for all three types of radiation; however, the slope increase was greater for photon radiation than for the JANUS neutron radiation.

The effects of the combination of Ara/c at its most protective time interval appeared to be additive when combined with WR-2721 for photon and for Fermilab neutrons. This was not the case for JANUS neutrons where there was no additional effect of Ara/c on the WR-2721 radioprotection. These results could be explained by the reduced effect of cell age distribution on survival after high LET neutron radiation.

In contrast to the effects of Ara/c on clonogenic cell survival, there was little or no effect of this treatment on the $LD50/_6$, nor was there any additive effect when Ara/c was combined with WR-2721. These results suggest that the survival of the intestinal stem cells is not closely associated with the survival of the animal. The dissociation of animal survival with stem cell survival was suggested by Lushbaugh (28); however, this view is not widely held. It is also of interest to note that the regimen of Ara/c treatment had no effect on the $LD50/_{30}$, nor was this regimen additive to WR-2721 changes in the $LD50/_{30}$, suggesting that the marrow stem cells do not respond or, at least, do not respond within the same time frame; however, data from other laboratories clearly indicate that the CFU-S can be recruited into rapid cycle following cytotoxic drug treatment such as hydroxyurea (29).

In summary, it has been shown that the potent S-phase cytotoxic agent, Ara/c, given 12 hours before both neutron and gamma irradiation protects cells from some degree of injury. This protection is seen at a time when the total number of cells per crypt is reduced to about half the normal number. These results strongly suggest that the number of clonogenic cells are few in number and that they are in a G_0 or an extended G_1 stage of the cell cycle. The most likely mechanism for the observed Ara/c induced radioprotection is through the redistribution of clonogenic cells mainly into the mid to late S-phase of the cell cycle. This radioprotection is additive to the protection by WR-2721 for low LET irradiation but little or no additivity was seen when combined with WR-2721 before high LET radiation. In spite of the radioprotection in intestinal stem cells, there was no effect of Ara/c on the $LD50/_6$ with or without WR-2721 (irrespective of the radiation source) suggesting the dissociation of stem cell survival from animal survival over the short term; however, long term survival must depend upon the survival of the stem cells.

SECTION 5

LIST OF REFERENCES

1. V.P. Bond, T.M. Fliedner, and J.O. Archambeau, Mammalian radiation lethality; a disturbance in cellular kinetics, Academic Press, New York, (1965).

2. H.R. Withers and M.M. Elkind, Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. Int. J. Radiat. Biol. 17, 261-267 (1970).

3. H.R. Withers and M.M. Elkind, Dose-survival characteristics of epithelial cells of mouse intestinal mucosa. Radiol. 91, 998-1000 (1968).

4. R.F. Hagemann and S. Lesher, Intestinal crypt survival and total and per crypt levels of proliferative cellularity following irradiation: Age response and animal lethality. Radiat. Res. 47, 159-167 (1971).

5. T.A. Boarder and N.M. Blackett, The proliferative status of intestinal epithelial clonogenic cells: Sensitivity to S phase specific cytotoxic agents. Cell Tissue Kinet. 9, 589-596 (1976).

6. K. Musada, H.R. Withers, K.A. Mason, and K.Y Chen, Single dose-response curves of murine gastrointestinal crypt stem cells. Radiat. Res. 69, 65-75 (1977).

7. R.F. Hagemann and S. Lesher, Intestinal cytodynamics: adductions from drug and radiation studies. In: Drugs and the cell cycle, Academic Press, New York, pp. 195-217 (1973).

8. J.R.M. Fry, A.B. Reiskin, W. Kisieleski, A. Fallese, and E. Staffeldt, Radiation effects and cell renewal in rodent intestine in radiation mortality in different mammalian species, In: Comparative Cellular and Species Radiosensitivity, (V.P. Bond and T. Sugahara, Eds.) Igaku Shoin Ltd., Tokyo, pp. 255-270 (1969).

9. C.S. Potten and J.H. Hendry, Differential regeneration of intestinal proliferative cells and cryptogenic cells after irradiation. Int. J. Radiat. Biol. 27, 413-424 (1975).

10. H. Cheng, and C.P. Leblond, Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. Am. J. Anat. 141, 461-480 (1974).

11. H. Cheng and C.P. Leblond, Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am. J. Anat. 141, 537-562 (1974).

12. W.R. Hanson, R.J.M. Fry and A.R. Sallese, Cytotoxic effects of colcemid or high specific activity tritiated thymidine on clonogenic cell survival in B6CF1 mice. Cell and Tissue Kinet. 12, 569-580 (1979).

13. D.E. Wimber, H. Quastler, O.L. Stein, and D.R. Wimber, Analysis of tritium incorporation into individual cells by autoradiography of squash preparations. J. Biophys. Biochem. Cytol. 8, 327-331 (1960).

14. C.S. Potten, C. Chadwick, K. Ijiri, S. Tsubonchi and W.R. Hanson, The recruitability and cell cycle state of intestinal stem cells. Int. J. of Cell Cloning 2, 126-140 (1984).

15. W.R. Hanson, D.L. Henninger and R.J.M. Fry, Time dependence of intestinal proliferative cell risk vs. stem cell risk to radiation or colcemid cytotoxicity following hydroxyurea, Int. J. Radiat. Oncology, Biol. and Phys. 5. 1685-1689 (1979).

16. W.R. Hanson and D.L. Boston, Cytosar-U (Ara-c) induced changes in mouse jejunal epithelial cell kinetics and radiosensitivity to gamma rays and fast neutrons, Int. J. Radiat. Oncology, Biol. and Physics 9, 515-521 (1983).

17. T.A. Phelps, Cytarabin (Ara-C) induced radioresistance of mouse jejunal stem cells following single or fractionated doses of radiation. Int. J. Radiat. Oncol. Biol. Phys. 6, 1671-1677 (1980).

18. L. Cohen and M. Awschalom, The cancer therapy facility at Fermi National Accelerator Laboratory, Batavia, Illinois: A preliminary report. Appl. Radiol. 5, 51-60 (1976).

19. C.P. Sigdestad, A.M. Connor, and R.M. Scott, The effect of S-2-(3-aminopropylamino) ethylphosphorothioxic acid (WR-2721) on intestinal crypt survival. I. 4 MeV X-rays. Radiat Res. 62, 267-275 (1975).

20. D. Grahn, E.J. Ainsworth, F.S. Williamson, and R.J.M. Fry, A program to study fission neutron-induced chronic injury in cells, tissues, and animal population, utilizing the JANUS reactor of the Argonne National Laboratory, In: Radiobiological Applications of Neutron Irradiation. IAEA, Vienna, pp.211-228 (1972).

21. T.R. Sweeney, A survey of compounds from the antiradiation drug development program of the U.S. Army Medical Research and Development Command, Walter Reed Army Institute of Research, Washington, D.C. (1979).

22. J.F. Ward, Chemical aspects of DNA radioprotection. In: Radioprotectors and Anticarcinogens, (O.F. Nygaard and M.G. Simic, Eds.), Academic Press, N.Y., pp. 73-85 (1983).

23. L. Milas, N. Hunter, H. Ito, E.L. Travis, and L.J. Peters, Factors influencing radioprotection of tumors by WR-2721. In: Radioprotectors and Anticarcinogens, (O.F. Nygaard and M.G. Simic Eds.) Academic Press, N.Y., pp. 695-718 (1983).

24. J.W. Harris, Cellular thiols in radiation and drug response: use of specific reagents. In: Radioprotectors and Anticarcinogens, (O.F. Nygaard and M.G. Simic, Eds.) Academic Press, N.Y., pp. 255-274 (1983).

25. J.E. Biaglow, M.E. Varnes, M. Astor, and J. Mitchell, Intracellular thiols: Involvement in drug metabolism and radiation response. In: Radioprotectors and Anticarcinogens, (O.F. Nygaard and M.G. Simic, Eds.), Academic Press, N.Y., pp. 203-236 (1983).

26. W.R. Hanson, D.L. Henninger, R.J.M. Fry and A.R. Sallese, The response of small intestinal stem cells in the mouse to drug and irradiation treatment, In: Cell Proliferation in the Gastrointestinal Tract. (Eds. Appleton, Sunter and Watson), Pitman Medical, pp. 198-212 (1980).

27. E.L. Gillette, H.R Withers, and I.F. Tannock, The age sensitivity of epithelial cells of mouse small intestine, Radiology 96, 639-643 (1970).

28. C.C. Lushbaugh, Theoretical and practical aspects of models explaining "gastrointestinal death" and other lethal radiation syndromes. In: Comparative Cellular and Species Radiosensitivity. (V.P. Bond and T. Sugahara, Eds.) Igaku Shoin Ltd., Tokyo, pp. 288-297 (1969).

29. E. Frindel, M. Guigon, D. Dumenil, and M.P. Fache, Stimulating factors and cell recruitment in murine bone marrow stem cells and EMT6 tumors. Cell Tissue Kinet. 11, 393-401 (1978).

DISTRIBUTION LIST

DEPARTMENT OF DEFENSE

ARMED FORCES RADIOBIOLOGY RSCH INST
ATTN: DEPUTY DIRECTOR
ATTN: DIRECTOR
10 CYS ATTN: MRCO
ATTN: SCIENTIFIC DIRECTOR
ATTN: TECHNICAL LIBRARY

ASST SECRETARY OF DEFENSE, PUBLIC AFFAIRS
ATTN: ASD(PA)

ASSISTANT SECRETARY OF DEFENSE
ATTN: ASD(HA)

DEFENSE INTELLIGENCE AGENCY
ATTN: RTS-2B

DEFENSE NUCLEAR AGENCY
ATTN: GC
ATTN: PAO
5 CYS ATTN: STRP
4 CYS ATTN: STTI-CA

DEFENSE TECHNICAL INFORMATION CENTER
12 CYS ATTN: DD

DEP UNDER SECY OF DEF FOR RSCH & ENGRG
ATTN: DUSDRE RSCH & ADV TECH

FIELD COMMAND DEFENSE NUCLEAR AGENCY
ATTN: FCTXE

FIELD COMMAND DNA DET 2
LAWRENCE LIVERMORE NATIONAL LAB
ATTN: FC-1

DEPARTMENT OF THE ARMY

HARRY DIAMOND LABORATORIES
ATTN: SLCIS-IM-TL TECH LIB

HQ DEPARTMENT OF THE ARMY
ATTN: DAAG-ESG-N, NTPR
ATTN: DASG-PSP-E

U S ARMY BALLISTIC RESEARCH LAB
ATTN: DRDAR-BLV-R J MALONEY

U S ARMY MEDICAL RSCH & DEV CMD
ATTN: SGRD-SD

U S ARMY NUCLEAR & CHEMICAL AGENCY
ATTN: MONA-ZB (C DAVIDSON)

USA MILITARY ACADEMY
ATTN: J CAMPBELL

WALTER REED ARMY MEDICAL CENTER
ATTN: LIBRARY

DEPARTMENT OF THE NAVY

NATIONAL NAVAL MEDICAL CENTER
ATTN: DEPT OF RADIOLOGY
ATTN: MEDICAL LIBRARY

NAVAL MEDICAL COMMAND
ATTN: MEDCOM-21
ATTN: NM&S-00
ATTN: NM&S-09

NAVAL OCEAN SYSTEMS CENTER
ATTN: TECH LIB

NAVAL SEA SYSTEMS COMMAND
ATTN: SEA-08 M MILES

NAVAL WEAPONS EVALUATION FACILITY
ATTN: CLASSIFIED LIBRARY

OFC OF THE DEPUTY CHIEF OF NAVAL OPS
ATTN: NOP 0455

DEPARTMENT OF THE AIR FORCE

AEROSPACE MEDICAL DIVISION, AFSC
ATTN: LIBRARY SCL-4

AIR FORCE INSTITUTE OF TECHNOLOGY/EN
ATTN: LIBRARY/AFIT/LDEE

AIR FORCE NUCLEAR TEST REVIEW
ATTN: SGPT COL GIBBONS

AIR FORCE WEAPONS LABORATORY, AFSC
ATTN: NT
ATTN: SUL

AIR UNIVERSITY LIBRARY
ATTN: AUL-LSE

U S AIR FORCE OCCUPATIONAL & ENV HEALTH LAB
ATTN: R2

DEPARTMENT OF ENERGY

DEPARTMENT OF ENERGY
ATTN: M MARELLI

DEPARTMENT OF ENERGY (CONTINUED)

DEPARTMENT OF ENERGY
ATTN: OMA, DP-22

DEPARTMENT OF ENERGY
ATTN: B CHURCH, HPD

DEPARTMENT OF ENERGY
ATTN: J THIESEN
ATTN: TECHNICAL INFO CTR

UNIVERSITY OF CALIFORNIA
LAWRENCE LIVERMORE NATIONAL LAB
ATTN: L ANSPAUGH
ATTN: L-53 TECH INFO DEPT LIBRARY
ATTN: Y NG

LOS ALAMOS NATIONAL LABORATORY
ATTN: F601 T DOWLER
ATTN: ITO D STILLMAN
ATTN: P WHALEN

OAK RIDGE NATIONAL LABORATORY
ATTN: C RICHMOND
ATTN: G KERR

OAK RIDGE NATIONAL LABORATORY
ATTN: T JONES

REYNOLDS ELECTRICAL AND ENGR CO, INC
ATTN: CIC
ATTN: LST
ATTN: W BRADY

OTHER GOVERNMENT

CANCER CENTER, NIH
ATTN: A KNUDSON

CENTRAL INTELLIGENCE AGENCY
ATTN: OFFICE OF MEDICAL SVCS

CONSUMER PRODUCT SAFETY COMMISSION
ATTN: M BLOOM
ATTN: P PRUESS

DEPARTMENT OF COMMERCE
ATTN: C KUYATT
ATTN: J HUBBELL
ATTN: M EHRLICH

DEPARTMENT OF HEALTH & HUMAN SERVICES
ATTN: OFC OF REGULATION REVIEW

DEPARTMENT OF HEALTH & HUMAN SVCS
ATTN: R MURPHY

DEPARTMENT OF LABOR
ATTN: S WEINER

DEPARTMENT OF TRANSPORTATION
ATTN: H L REIGHARD

DEPT OF HEALTH & HUMAN SERVICES
ATTN: C SILVERMAN
ATTN: G JOHNSON
ATTN: J VILLFORTH

ENVIRONMENTAL PROTECTION AGENCY
ATTN: P MAGNO
ATTN: T THORSLUND

ENVIRONMENTAL PROTECTION AGENCY
ATTN: N NELSON

FEDERAL EMERGENCY MANAGEMENT AGENCY
ATTN: C SIEBENTRITT
ATTN: H TOVEY
ATTN: OFC OF RSCH/NP H TOVEY

LIBRARY OF CONGRESS
ATTN: SCIENCE & TECHNOLOGY DIV

NASA HEADQUARTERS
ATTN: P RAMBAUT

NATIONAL CANCER INSTITUTE, NIH
ATTN: B WACHOLZ
ATTN: G BEEBE
ATTN: V ZEVE

NATIONAL CANCER INSTITUTE, NIH
ATTN: C LAND
ATTN: J FRAUMENI
ATTN: W BLOT

NATIONAL CANCER INSTITUTE, NIH
ATTN: J GART

NATIONAL CANCER INSTITUTE, NIH
ATTN: A RABSON
ATTN: D PISTENMAA
ATTN: J WYNGAARDEN

NATIONAL INST FOR OCCUP SAFETY & HEALTH
ATTN: W MURRAY

NATIONAL INSTITUTES OF HEALTH
ATTN: LIBRARY (ACQ UNIT)

NATIONAL LIBRARY OF MEDICINE, NIH
ATTN: LIBRARY

NATIONAL SCIENCE FOUNDATION
ATTN: P HARRLMAN

OFFICE ON SMOKING & HEALTH
ATTN: J PINNEY

OTHER GOVERNMENT (CONTINUED)

U S NUCLEAR REGULATORY COMMISSION
ATTN: R WHIPP FOR F ARSENAULT
ATTN: R WHIPP FOR R MINOGUE

U S PUBLIC HEALTH SERVICE
ATTN: LIBRARY

VETERANS ADMIN MEDICAL CENTER
ATTN: K LEE

VETERANS ADMIN MEDICAL CENTER
ATTN: D MCGREGOR

VETERANS ADMIN MEDICAL CENTER
ATTN: C TESSMER

VETERANS ADMIN WADSWORTH HOSPITAL CTR
ATTN: T MAKINODAN

VETERANS ADMINISTRATION HOSPITAL
ATTN: R YALOW

VETERANS ADMINISTRATION-RO
ATTN: DIRECTOR

DEPARTMENT OF DEFENSE CONTRACTORS

ADVANCED RESEARCH & APPLICATIONS CORP
ATTN: R ARMISTEAD

BDM CORP
ATTN: J BRADDOCK

JAYCOR
ATTN: A NELSON INFO SYS DIV

KAMAN TEMPO
ATTN: DASIAC

LOUISIANA UNIV SCH OF MED, SHREVEPORT
ATTN: LIBRARY

NATIONAL ACADEMY OF SCIENCES
ATTN: C ROBINETTE
ATTN: S JABLON

NEBRASKA, UNIVERSITY OF
ATTN: LIBRARY

NORTHROP CORP
ATTN: Z SHANFIELD

OHIO STATE UNIVERSITY
ATTN: LIBRARY

PACIFIC-SIERRA RESEARCH CORP
ATTN: H BRODE, CHAIRMAN SAGE

R & D ASSOCIATES
ATTN: C K B LEE

R & D ASSOCIATES
ATTN: A DEVERILL

RADIATION RESEARCH ASSOCIATES, INC
ATTN: N SCHAEFFER

RAND CORP
ATTN: P DAVIS
ATTN: TECH LIBRARY

RAND CORP
ATTN: B BENNETT

SCIENCE APPLICATIONS INTL CORP
ATTN: J GOETZ
ATTN: J MCGAHAN

SCIENTIFIC INFORMATION SERVICES, INC
ATTN: LIBRARY

FOREIGN

CANADIAN EMBASSY
ATTN: LIBRARY

EDF - RETN 1
ATTN: LIBRARY

INDIAN COUNCIL OF MEDICAL RSCH
ATTN: A TASKAR

JAPAN-HAWAII CANCER STUDY
ATTN: G GLOBER

MAURICE DELPLA
ATTN: M DELPLA

MCGILL UNIVERSITY
ATTN: R OSEASOHN

PRESIDENTE UMBERTO COLOMBO
ATTN: LIBRARY

PUERTO RICO SCH OF MEDICINE, UNIV OF
ATTN: LIBRARY

UNITED KINGDOM SCIENTIFIC MISSION
ATTN: MIL LIASION FOR DR RIDLEY

DIRECTORY OF OTHER

APPLIED SCIENCES LABORATORY
ATTN: B ADCOCK
ATTN: J AUXIER

BROOKHAVEN NATIONAL LABORATORY
ATTN: A B BRILL, MEDICAL DEPT
ATTN: E CRONKITE, MEDICAL DEPT
ATTN: E LASSARD, ENVIRON DEPT
ATTN: M BENDER, MEDICAL DEPT
ATTN: TECHNICAL LIBRARY
ATTN: V BOND

DIRECTORY OF OTHER (CONTINUED)

CALIFORNIA INSTITUTE OF TECHNOLOGY
ATTN: E LEWIS
ATTN: R CHRISTY

CHICAGO, UNIVERSITY OF
ATTN: P MEIER

COLUMBIA UNIVERSITY
ATTN: A BLOOM
ATTN: LIBRARY

COORDINATION & INFORMATION CTR
ATTN: C/O REECD

CORNELL UNIVERSITY
ATTN: W FEDERER

HARVARD SCHOOL OF PUBLIC HEALTH
ATTN: J BAILOR
ATTN: LIBRARY
ATTN: R REED

HARVARD SCHOOL OF PUBLIC HEALTH
ATTN: B MACMAHON

HARVARD UNIVERSITY
ATTN: W COCHRAN

HAWAII, UNIVERSITY OF
ATTN: Y MATSUMOTO

INDIANA UNIVERSITY
ATTN: F PUTNAM

IOWA STATE UNIVERSITY
ATTN: T BANCROFT

JOHNS HOPKINS UNIVERSITY
ATTN: A KIMBALL
ATTN: T MITCHELL

KINGSTON HOSPITAL
ATTN: K JOHNSON

MEMORIAL HOSP FOR CANCER & ALLIED DISEASES
ATTN: P LIEBERMAN

MEMORIAL SLOAN-KETTERING CANCER CENTER
ATTN: J LAUGHLIN
ATTN: P MARKS

MERCK, SHARP & DOHME INTL
ATTN: A BEARN

MICHIGAN MEDICAL SCHOOL, UNIV OF
ATTN: J NEEL

MICHIGAN, UNIVERSITY OF
ATTN: R CORNELL

MICHIGAN, UNIVERSITY OF
ATTN: F MOORE

MINNESOTA, UNIVERSITY OF
ATTN: J BEARMAN
ATTN: L SCHUMAN
ATTN: LIBRARY

NATL COUNCIL ON RADIATION
ATTN: W SINCLAIR

NEW MEXICO, UNIV OF
ATTN: C KEY
ATTN: R ANDERSON

NEW YORK UNIV MEDICAL CENTER
ATTN: N NELSON

NEW YORK UNIVERSITY
ATTN: A UPTON
ATTN: B POSTERNACK
ATTN: LIBRARY

NORTHWESTERN UNIVERSITY
ATTN: H CEMBER

OAK RIDGE ASSOCIATED UNIVERSITIES
ATTN: D LUSHBAUGH
ATTN: E TOMPKINS
ATTN: J TOTTER

OHIO STATE LIBRARY
ATTN: LIBRARIAN

OKLAHOMA, UNIVERSITY OF
ATTN: P ANDERSON

OREGON, UNIVERSITY OF
ATTN: B PIROFSKY

PACIFIC NORTHWEST LABORATORY
ATTN: J STROUD
ATTN: S MARKS

PENNSYLVANIA UNIV HOSPITAL
ATTN: S BAUM

PENNSYLVANIA, UNIV OF
ATTN: P NOWELL

PITTSBURGH, UNIV OF
ATTN: E RADFORD
ATTN: LIBRARY

PITTSBURGH, UNIVERSITY OF
ATTN: N WALD
ATTN: R SELTZER

DIRECTORY OF OTHER (CONTINUED)

ROCHESTER UNIV MEDICAL CTR
ATTN: C ODOROFF
ATTN: G CASARETT

ROCHESTER, UNIVERSITY OF
ATTN: L HEMPELMANN

RUSH PRESBYTERIAN-ST LUKE'S MED CTR
ATTN: W HANSON

SAINT FRANCIS HOSPITAL
ATTN: R BLAISDELL

SOUTH CAROLILNA, MEDICAL UNIV OF
ATTN: P LIU

SOUTHERN CALIFORNIA, UNIV OF
ATTN: J BIRREN

SOUTHERN ILLINOIS UNIVERSITY
ATTN: DOCUMENTS CTR

STANFORD UNIV MEDICAL CENTER
ATTN: J BROWN

STANFORD UNIVERSITY
ATTN: L MOSES

STANFORD UNIVERSITY HOSPITAL
ATTN: D DORFMAN

TACOMA PUBLIC LIBRARY
ATTN: LIBRARIAN

TEXAS A & M UNIVERSITY
ATTN: R STONE

TEXAS AT AUSTIN, UNIV OF
ATTN: H SUTTON

TEXAS, UNIVERSITY OF
ATTN: C S COOK

TEXAS, UNIVERSITY OF
ATTN: R STALLONES

TEXAS, UNIVERSITY OF
ATTN: W SUTOW

TEXAS, UNIVERSITY OF
ATTN: G TAYLOR

UTAH, UNIVERSITY OF
ATTN: DOC DIVISION

VANDERBILT UNIVERSITY
ATTN: R QUINN

VERMONT, UNIVERSITY OF
ATTN: DIRECTOR OF LIBRARIES

WASHINGTON, UNIVERSITY OF
ATTN: D THOMPSON

WASHINGTON, UNIVERSITY OF
ATTN: A MOTULSKY

WISCONSIN, UNIVERSITY OF
ATTN: J CROW

YALE UNIVERSITY SCH OF MEDICINE
ATTN: J MEIGS
ATTN: LIBRARY